REMARKS

Entry of the foregoing, reexamination and further and favorable reconsideration of the subject application in light of the following remarks, pursuant to and consistent with 37 C.F.R. § 1.112, are respectfully requested.

By the foregoing amendment, the specification has been amended in numerous locations as discussed *infra*. Support for the amendments to the specification can be found throughout the originally filed application. Additionally, original claims 1-10 have been canceled without prejudice or disclaimer to the subject matter recited therein and new claims 11-20 have been added. New claims 11-19 correspond essentially to original claims 1-9. In addition to the language recited in original claims 1, the term "detecting" in step (d) has been replaced with the phrase "determining the existence or absence of . . ." and step (e) has been included. Support for this language can be found on at least page 27, line 33 to page 28, line 5 of the specification. In addition to the language recited in original claim 9, claim 19 further recite step (c). Support for step (c) in claim 19 can be found, for example, in original claim 10 and on page 28, lines 34-36 of the specification. New claim 20 depends on claim 19. Support for new claim 20 can be found on at least page 25, lines 13-15 of the Specification.

With the Official Action mailed on December 20, 2002, the Examiner attached an initialed copy of the PTO-1449 Form submitted with the Information Disclosure Statement filed on September 9, 2002. However, in the Information Disclosure Statement filed on September 9, 2002, applicants cited more than the foreign document listed on the PTO-

1449 Form. More specifically, on page 2 of the Information Disclosure Statement filed on September 9, 2002, applicants also directed the Examiner's attention to co-pending Application Serial No. 09/549,949.[1] Despite applicants' request, an initialed copy of the second page of the Information Disclosure Statement citing the co-pending application was not returned to applicants. Accordingly, applicants once again request that the Examiner initial the box on the second page of the Information Disclosure Statement and return a copy to applicants. For the Examiner's convenience, a copy of the September 9, 2002 Information Disclosure Statement is attached hereto.

Additionally, with regard to co-pending Application Serial No. 09/549,949, the Examiner's attention is directed to the fact that such application has recently issued as U.S. Patent No. 6,541,226. Hence, an information disclosure statement, and corresponding PTO-1449 Form, listing this issued patent is attached hereto.

On page 2 of the Official Action the Examiner alleges that the specification contains "some unclear, inexact or verbose terms . . . ." Applicants disagree with the Examiner's statements in this regard. However, to advance prosecution, and not to acquiesce to the Examiner's allegations, applicants have amended the specification throughout pursuant to the Examiner's comments. Such amendments are not intended to limit any aspect of the disclosed invention. Moreover, no new matter has been added. The amendments to the specification in this regard are self-explanatory. It is noted with particularity, however, that the description of gel lanes in Figures 4, 6, 9, 10, 12 and 13 have been amended to

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[1] A copy of such co-pending application was also submitted.

clarify that the particular oligonucleotide or heat treatment was used. As indicated by the Examiner, "it is clear from the description of the experiments further in the specification that they were used." It is also particularly noted that the brief description of the drawings have been amended so as to contain separate designations for the figures designated as A, B, C, etc. With regard to the description for Figure 10C, support for such amendment can be found on at least page 17, lines 27-29 and page 36, lines 11-12 of the specification. Moreover, in accordance with the amendments to the brief description of the drawings, included herewith is a Request for Approval of Drawing Changes regarding Figures 1, 2, 3, 4, 6, 9, 10, 12 and 13.

Claims 1-9 have been objected to for ending in a comma as opposed to a period. Claims 1-9 have been canceled by the foregoing amendment. Thus, this rejection is rendered moot. It is noted that the newly added claims all end with a period. Accordingly, withdrawal of the objection to the claims is respectfully requested.

Claims 1-8 and 10 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for purportedly failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 1-8 and 10 have been canceled by the foregoing amendment. Thus, this rejection is rendered moot.

To the extent that rejection under 35 U.S.C. § 112, second paragraph, may apply to the newly added claims, applicants respectfully traverse the rejection. As described above, step (d) of claim 11 recites "determining the existence or absence of an oligonucleotide probe . . ." and step (e) involves judging whether or not SNP exists in the DNA region

complimentary to the oligonucleotide probe in the target double strand DNA. Moreover, none of the claims recited the language objected to with regard to original claim 10.

In view of the above, the Examiner is respectfully requested to withdraw the rejection under 35 U.S.C. § 112, second paragraph.

Claims 9 and 10 have been rejected under 35 U.S.C. §§ 102(a) & (e) as purportedly being anticipated by U.S. Patent No. 6,200,812 B1 issued to Pati et al. Claims 9 and 10 have also been rejected under 35 U.S.C. § 102(b) as purportedly being anticipated by U.S. Patent No. 6,074,853 issued to Pati et al. Further, claims 9 and 10 have been rejected under 35 U.S.C. § 102(e) as purportedly being anticipated by U.S. Patent No. 6,335,164 B1 issued to Kigawa et al. Each of these rejections are respectfully traversed.

Claims 9 and 10 have been canceled by the foregoing amendment. Thus, this rejection is rendered moot. However, to the extent that the Examiner may consider these rejections to apply to the currently pending claims, applicants traverse each of these rejections for at least the following reasons.

The Federal Circuit has held that for prior art to be anticipatory, every element of the claimed invention must be disclosed in a single item of prior art in the form literally defined in the claim. *See, e.g., Hybritech, Inc. v Monoclonal Antibodies, Inc.*, 213 U.S.P.Q. 81, 90 (Fed. Cir. 1986). This requirement for anticipation has clearly not been met with respect tot he currently pending claims of the present application.

The Examiner has indicated that the '812 Pati et al. patent and the '853 Pati et al. patent disclose a kit including DNA probes, recombinases, buffers, and ATP. With regard

to the '164 Kigawa et al. patent, the Examiner has indicated that such patent discloses a kit comprising a Rec A-like recombinase, appropriate co-factors, a heterologous and homologous probes, and a washing solution.

Currently pending claims 19 and 20, which are directed to a kit, recite that the kit comprises "a reagent removing the homologous recombinant protein." None of the patents cited by the Examiner — the '812 Pati et al. patent, the '853 Pati et al. patent, and the '164 Kigawa et al. patent — include such a reagent. Accordingly, none of the cited patents disclose each and every element of the claimed invention.

In light of the above, the Examiner is respectfully requested to withdraw each of the rejections under 35 U.S.C. § 102.

Finally, the Examiner has provisionally rejected claims 9 and 10 under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claim 9 of co-pending Application Serial No. 09/549,949. This rejection is respectfully traversed.

Claims 9 and 10 have been canceled by the foregoing amendment. Thus, this rejection is rendered moot. However, to the extent that the Examiner may consider these rejections to apply to the currently pending claims, applicants traverse each of these rejections for at least the following reasons.

The analysis employed in an obviousness-type double patenting rejection parallels the guidelines for a 35 U.S.C. § 103 obviousness determination. *See* M.P.E.P. § 804.

As mentioned *supra*, co-pending Application Serial No. 09/549,949 has recently issued as U.S. Patent No. 6,541,226. Neither claim 9 of co-pending Application Serial No. 09/549,949, nor any of the claims in the '226 patent, render obvious the kit claims — claims 19 and 20 — of the present application. As discussed *supra*, claims 19 and 20 recite that the kit comprises "a reagent removing the homologous recombinant protein." However, claim 9 of co-pending Application Serial No. 09/549,949, or any one of the claims in the '226 patent, fails to teach or suggest the inclusion of such a reagent in the kit. Accordingly, claims 19 and 20 are not rendered obvious by claim 9 of co-pending Application Serial No. 09/549,949, or any one of the claims in the '226 patent.

In light of the above, the Examiner is respectfully requested to withdraw the obviousness-type double patenting rejection.

From the foregoing, further and favorable action in the form of a Notice of Allowance is respectfully requested and such action is earnestly solicited.

In the event that there are any questions relating to this Amendment and Reply, or the application in general, it would be appreciated if the Examiner would telephone the

undersigned attorney concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

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Date: June 17, 2003

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Attachment to Amendment and Reply Dated June 20, 2003

Marked-Up Copy of Specification

Paragraph beginning at Page 1, line 9

Differences ~~of~~ in various phenotypes of ~~human~~ humans, including disease, are known to be derived from the differences ~~of~~ in DNA nucleotide sequence in an individual ~~genome, which~~ genome. This difference is called single nucleotide polymorphism (SNP). SNPs are found widely in human genome of about 3 billion base pairs and the total number of SNPs is not less than 3 millions. Thus, SNPs can be the DNA markers having exceptionally higher density that known DNA markers such as RFLP (restriction fragment length polymorphism) and STR (microsatellite), which are conventional DNA markers. Therefore, high-precision analysis, which has been impossible using conventional DNA markers, is possible by using SNPs, and it is hoped that SNP can be applied to detection of disease genes, determination of disease sensitivity, and development of pharmaceutical.

Paragraph beginning at Page 2, line 11

However, there is ~~the~~ a problem ~~for~~ with this technique because ~~in which~~ the region for searching is from 200 to 300 bp ~~for this method~~ and the detection fails when the region is not more than that.

Paragraph beginning at Page 3, line 18 and ending on Page 4, line 5

The present inventors have intensively been studying homologous recombination of DNA *in vivo*. As a result of the study, the present inventors revealed that *E coli* RecA protein involved in homologous recombination can make triple strand DNA without long homologous region and that the triple strand DNA becomes unstable by heat when a pair of mismatch exists in one double strand DNA among triple strand DNA. Then, the present inventors reminded a following method for detecting DNA polymorphism. First, an oligonucleotide probe complementary to one strand of test DNA region to examine is prepared and hybridized to the test DNA region using a homologous recombination protein. After the formation of triple strand DNA in the test DNA region, the homologous recombination protein is removed. When polymorphism ~~is existed~~ exists in the test DNA region, mismatch nucleotide pair occurs between the oligonucleotide probe and one strand of the test DNA region which makes the structure of triple strand DNA unstable to heat compared to the test DNA region in which no polymorphism exists. If the triple strand DNA is treated with heat, the oligonucleotide probe is released from unstable triple strand DNA in which mismatch exists. Therefore, the present inventors considered that the existence of polymorphism in test DNA region could be detected by detecting oligonucleotide probe hybridized to the test DNA region.

Paragraph beginning at Page 4, line 6

The present inventors investigated whether the detection of DNA polymorphism was actually possible by using the above-mentioned method. At first, the present inventors examined the condition in which a test DNA region including polymorphism and an oligonucleotide which was a probe for the detection of polymorphism could form stable triple strand DNA through a homologous recombination protein. As a result, it was revealed that triple strand DNA could be formed if the length of the oligonucleotide probe was not less than 40 nucleotides. Next, the present inventors examined the stability of triple strand DNA ~~to heat~~, which comprises an oligonucleotide completely complementary to one strand of a test DNA region or an oligonucleotide including one mismatch and the test double DNA, to heat. As a result, the triple strand DNA formed when the completely complementary oligonucleotide was more stable than that containing an oligonucleotide including one mismatch. Thus, the difference of thermostability between them was clear. It was revealed that the stability of the triple strand DNA was markedly affected by mismatch derived from even one nucleotide mutation in a DNA strand. Therefore, it is considered that the heat treatment of triple strand DNA makes oligonucleotide having mismatch release from target DNA and makes the structure of triple strand DNA collapse. Using the above-mentioned result, precise detection of polymorphism existing in the test DNA is possible by detecting oligonucleotide that forms triple strand DNA with target DNA even after heat treatment of the triple strand DNA that is formed using homologous recombination protein.

Paragraph beginning at Page 5, line 11 and ending on Page 6, line 21

The present invention relates to a new method which does not require long DNA region for searching and can detect DNA polymorphism with high specificity and efficiency. More specifically, the present invention provides the ~~followings~~ following:

[1] A method for detecting a DNA polymorphism in a double strand DNA, said method comprising the steps of (a) to (d) below:

(a) contacting (i) a double strand DNA comprising a test polymorphic site, (ii) an oligonucleotide probe that hybridizes to a region comprising said polymorphic site in said double strand DNA, and (iii) a homologous recombination protein under reaction conditions where a triple strand DNA complex is formed,

(b) removing the homologous recombination protein from the triple strand DNA complex formed in the step (a), ~~thereby generating a triple strand DNA~~ thereby generating a triple strand DNA,

(c) conducting heat treatment of the triple strand DNA generated by removing the homologous recombination protein, under conditions where the oligonucleotide probe is released from said triple strand DNA, when the test polymorphic site in the double strand DNA is not complementary to a corresponding site in said oligonucleotide probe,

(d) detecting an oligonucleotide probe that binds to the double strand DNA to form the triple strand DNA,

[2] The method of [1], wherein the double strand DNA comprising a test polymorphic site has a DNA terminus,

[3] The method of [2], wherein the test polymorphic site is located within 20 bases from the DNA terimus,

[4] The method of [1], wherein the length of the oligonucleotide probe is from 20 to 120 bases,

[5] The method of [1], wherein the homologous recombination protein is a RecA protein from *E. coli*,

[6] The method of [1], wherein, in the step (a), a nucleotide triphosphate is added to the reaction system,

[7] The method of [1], wherein, in the step (b), the homologous recombination protein is removed by conducting protein degradation enzyme treatment,

[8] The method of [7], wherein the protein degradation enzyme is proteinase K,

[9] A lit for detecting a polymorphism in a double strand DNA comprising a test polymorphic site, said kit comprising the following components: (a) an oligonucleotide probe that hybridizes to the double strand DNA comprising the test polymorphic site and (b) a homologous recombination protein,

[10] A kit of [9], further comprising at least one selected from the group consisting of (i) a reagent removing the homologous recombination protein, (ii) nucleotide triphosphate, and (iii) a buffering agent.

Paragraph beginning at Page 6, line 24

~~Figure 1~~ Figures 1A-1C show the following: ~~Upper part~~ Figure 1A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide ~~consisting~~ containing sequence complementary to the DNA. ~~The left (A) of lower part~~ Figure 1B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was electrophoresed. ~~The right (B) of lower part~~ Figure 1C is the photograph of staining gel with ethidium bromide after electrophoresis. Each lane is as follows:

Lane M: DNA size marker (The left in the figure indicates size. This size marker is λDNA which was cut by restriction enzyme Hind III and whose 5'-terminal was labeled with ^{32}P using T4 Polynucleotide kinase and [γ-^{32}P] ATP.

Paragraph beginning at Page 7, line 17

~~Figure 2~~ Figures 2A-2C show the following: ~~Upper part~~ Figure 2A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide ~~consisting~~ containing sequence complementary to the DNA. ~~The left (A) of lower part~~ Figure 2B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was electrophoresed. ~~The right (B) of lower part~~ Figure 2C is the photograph of staining gel with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1: The reaction was performed in the same manner of lane 1 of Figure ~~1(A)~~ 1B.

Paragraph beginning at Page 7, line 34 and ending at Page 8, line 6

~~Figure 3~~ Figures 3A-3C show the following ~~Upper part~~ Figure 3A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide ~~consisting~~ containing sequence complementary to the DNA. ~~The left (A) of lower part~~ Figure 3B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was electrophoresed. ~~The right (B) of lower part~~ Figure 3C is the photograph of staining gel with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1: The reaction was performed in the same manner of lane 1 of Figure ~~1(A)~~ 1B.

Paragraph beginning at Page 8, line 24

~~Figure 4~~ Figures 4A-4C show the following ~~Upper part~~ Figure 4A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide ~~consisting~~ containing sequence complementary to the DNA. ~~The left (A) of lower part~~ Figure 4B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was electrophoresed. ~~The~~

~~right (B) of lower part~~ Figure 4C is the photograph of staining gel with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1: The reaction was performed in the same manner of lane 1 of Figure ~~1(A)~~ 1B in which ~~without using~~ labeled oligonucleotide 11 that had the 5'-terminal sequence extending 20 per of oligonucleotide 1 was used.

Paragraphs beginning at Page 9, line 17 and ending at Page 11, line 20

~~Figure 6~~ Figures 6A-6C show the following: ~~Upper part~~ Figure 6A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide ~~consisting~~ containing sequence complementary to the DNA. "G" or "C" on the oligonucleotide indicates the types of the nucleotide located at corresponding base pair of the target DNA shown just described above in character in the figure. ~~The left (A) of lower part~~ Figure 6B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was electrophoresed. ~~The right (B) of lower part~~ Figure 6C is the photograph of staining gel with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1: The heat treatment was conducted at 25°C for 10 minutes using oligonucleotide 1.

Lane 2: The reaction was performed in the same manner of lane 1 in which ~~without conducting~~ the heat treatment was conducted at 65°C for 10 minutes.

Lane 3: The reaction was performed in the same manner of lane 1 in which ~~without conducting~~ the heat treatment was conducted at 70°C for 10 minutes.

Lane 4: The reaction was performed in the same manner of lane 1 in which ~~without conducting~~ the heat treatment was conducted at 75°C for 10 minutes.

Lane 5: The reaction was performed in the same manner of lane 1 in which ~~without conducting~~ the heat treatment was conducted at 80°C for 10 minutes.

Lane 6: The reaction was performed in the same manner of lane 1 in which ~~without conducting~~ the heat treatment was conducted at 85°C for 10 minutes.

Lane 7: The reaction was performed in the same manner of lane 1 in which ~~without conducting~~ the heat treatment was conducted at 90°C for 10 minutes.

Lane 8: The reaction was performed in the same manner of lane 1 in which ~~without conducting~~ the heat treatment was conducted at 95°C for 10 minutes.

Lane 9: The reaction was performed in the same manner of lane 1 in which ~~without using~~ oligonucleotide 16 was used.

Lane 10 The reaction was performed in the same manner of lane 2 in which ~~without using~~ oligonucleotide 16 was used.

Lane 11: The reaction was performed in the same manner of lane 3 in which ~~without using~~ oligonucleotide 16 was used.

Lane 12: The reaction was performed in the same manner of lane 4 in which ~~without using~~ oligonucleotide 16 was used.

Lane 13: The reaction was performed in the same manner of lane 5 in which ~~without using~~ oligonucleotide 16 was used.

Lane 14: The reaction was performed in the same manner of lane 6 in which ~~without using~~ oligonucleotide 16 was used.

Lane 15: The reaction was performed in the same manner of lane 7 in which ~~without using~~ oligonucleotide 16 was used.

Lane 16: The reaction was performed in the same manner of lane 8 in which ~~without using~~ oligonucleotide 16 was used.

Lane 17: After the reaction mixture including 1 pmol labeled oligonucleotidel, 10 pmol unlabeled oligonucleotide 2, 100 ng M13 mp18 ssDNA, 4.8 mM ATP-γS, 30 mM Tris acetate (pH 7.2), and 20 mM magnesium acetate was incubated at 37°C for 30 minutes, 0.5% (W/Vol) SDS and 0.7 mg/ml proteinase K was added to the mixture. Then, the mixture was incubated at 37°C for 30 minutes. Subsequent reaction was performed in the same manner of lane 1.

Lane 18: The reaction was performed in the same manner of lane 17 in which ~~without treating~~ the reaction mixture was treated with heat at 65°C for 10 minutes.

Lane 19: The reaction was performed in the same manner of lane 17 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 20: The reaction was performed in the same manner of lane 17 in which ~~without treating~~ the reaction mixture was treated with heat at 75°C for 10 minutes.

Lane 21: The reaction was performed in the same manner of lane 17 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 22: The reaction was performed in the same manner of lane 17 in which ~~without treating~~ the reaction mixture was treated with heat at 85°C for 10 minutes.

Lane 23: The reaction was performed in the same manner of lane 17 in which ~~without treating~~ the reaction mixture was treated with heat at 90°C for 10 minutes.

Paragraph beginning at Page 11, line 21

Figure 7 shows the intensity of the signal from the labeled oligonucleotide, ~~was~~ measured by BAS2000 Image analyzer, and the result. Longitudinal axis indicates the temperature (°C). Triangles show the result of lane 1 to 8 while black triangles show the result of lane 9 to 16. Circles show the result of lane 17 to 23.

Paragraph beginning at Page 11, line 28

Figure 8 schematically shows the relation of the position between target DNA (PCR product) used for the experiment and the oligonucleotide ~~consisting~~ containing sequence complementary to the DNA. "A", "T", "G" or "C" on the oligonucleotide indicates the types of the nucleotide located at corresponding base pair of the target DNA shown just described above in character in the figure.

Paragraphs beginning at Page 11, line 34 and ending at Page 15, line 30

~~Figure 9~~ Figures 9A-9B show the following: ~~(A)~~ Figure 9A is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was electrophoresed. ~~The right (B) of lower part~~ Figure 9B is the photograph of staining gel corresponding to ~~(A)~~ Figure 9A with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1: The reaction was performed in the same manner of lane 1 of ~~Figure 6~~ Figures 6B-6C in which ~~without using~~ PCR product was used as the target DNA, and oligonucleotide 3 was used.

Lane 2: The reaction was performed in the same manner of lane 1 in which ~~without using~~ oligonucleotide 17 was used.

Lane 3: The reaction was performed in the same manner of lane 1 in which ~~without using~~ oligonucleotide 18 was used.

Lane 4: The reaction was performed in the same manner of lane 1 in which ~~without using~~ oligonucleotide 19 was used.

Lane 5: The reaction was performed in the same manner of lane 1 in which ~~without treating~~ the reaction mixture was treated with heat at 70° for 10 minutes after unused oligonucleotide was removed by S-400 spin column.

Lane 6: The reaction was performed in the same manner of lane 2 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 7: The reaction was performed in the same manner of lane 3 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 8: The reaction was performed in the same manner of lane 4 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 9: The reaction was performed in the same manner of lane 1 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 10: The reaction was performed in the same manner of lane 2 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 11: The reaction was performed in the same manner of lane 3 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 12: The reaction was performed in the same manner of lane 4 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 13: The reaction was performed in the same manner of lane in which ~~without using~~ the PCR Product (b) that was obtained by the PCR using primer 3 and primer 2 was used as the target DNA.

Lane 14: The reaction was performed in the same manner of lane 13 in which ~~without using~~ oligonucleotide 17 was used.

Lane 15: The reaction was performed in the same manner of lane 13 in which ~~without using~~ oligonucleotide 18 was used.

Lane 16: The reaction was performed in the same manner of lane 13 in which ~~without using~~ oligonucleotide 19 was used.

Lane 17: The reaction was performed in the same manner of lane 13 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 18: The reaction was performed in the same manner of lane 14 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 19: The reaction was performed in the same manner of lane 15 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 20: The reaction was performed in the same manner of lane 16 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 21: The reaction was performed in the same manner of lane 13 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 22: The reaction was performed in the same manner of lane 14 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 23: The reaction was performed in the same manner of lane 15 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 24: The reaction was performed in the same manner of lane 16 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 25: The reaction was performed in the same manner of lane 1 in which ~~without using~~ the PCR Product (c) that was obtained by the PCR using primer 4 and primer 2 was used as the target DNA.

Lane 26: The reaction was performed in the same manner of lane 25 in which ~~without using~~ oligonucleotide 17 was used.

Lane 27: The reaction was performed in the same manner of lane 25 in which ~~without using~~ oligonucleotide 18 was used.

Lane 28: The reaction was performed in the same manner of lane 25 in which ~~without using~~ oligonucleotide 19 was used.

Lane 29: The reaction was performed in the same manner of lane 25 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 30: The reaction was performed in the same manner of lane 26 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 31: The reaction was performed in the same manner of lane 27 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 32: The reaction was performed in the same manner of lane 28 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 33: The reaction was performed in the same manner of lane 25 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 34: The reaction was performed in the same manner of lane 26 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 35: The reaction was performed in the same manner of lane 27 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 36: The reaction was performed in the same manner of lane 28 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 37: The reaction was performed in the same manner of lane 1 in which ~~without using~~ the PCR Product (d) that was obtained by the PCR using primer 5 and primer 2 was used as the target DNA.

Lane 38: The reaction was performed in the same manner of lane 37 in which ~~without using~~ oligonucleotide 17 was used.

Lane 39: The reaction was performed in the same manner of lane 37 in which ~~without using~~ oligonucleotide 18 was used.

Lane 40: The reaction was performed in the same manner of lane 37 in which ~~without using~~ oligonucleotide 19 was used.

Lane 41: The reaction was performed in the same manner of lane 37 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 42: The reaction was performed in the same manner of lane 38 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 43: The reaction was performed in the same manner of lane 39 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 44: The reaction was performed in the same manner of lane 40 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 45: The reaction was performed in the same manner of lane 37 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 46: The reaction was performed in the same manner of lane 38 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 47: The reaction was performed in the same manner of lane 39 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 48: The reaction was performed in the same manner of lane 40 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Paragraphs beginning at Page 15, line 31 and ending at Page 17, line 18

~~Figure 10~~ Figures 10A-10C show the following: ~~Upper part~~ Figure 10A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide ~~consisting~~ containing sequence complementary to the DNA. "G" on the oligonucleotide indicates the relative position of mutation. ~~The lower part~~ Figure 10B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was electrophoresed. Figure 10C is the photograph of staining gel corresponding to Figure 10B with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1: The reaction was performed in the same manner of lane 1 of ~~Figure 6~~ Figures 6B-6C of Example 6 in which ~~without using~~ oligonucleotide 20 was used.

Lane 2: The reaction was performed in the same manner of lane 1 in which ~~without using~~ ologonucleotide 21 was used.

Lane 3: The reaction was performed in the same manner of lane 1 in which ~~without using~~ ologonucleotide 22 was used.

Lane 4: The reaction was performed in the same manner of lane 1 in which ~~without using~~ ologonucleotide 23 was used.

Lane 5: The reaction was performed in the same manner of lane 1 in which ~~without using~~ ologonucleotide 24 was used.

Lane 6: The reaction was performed in the same manner of lane 1 in which ~~without using~~ ologonucleotide 25 was used.

Lane 7: The reaction was performed in the same manner of lane 1 in which ~~without using~~ ologonucleotide 26 was used.

Lane 8: The reaction was performed in the same manner of lane 1 in which ~~without using~~ ologonucleotide 27 was used.

Lane 9: The reaction was performed in the same manner of lane 1 in which ~~without using~~ ologonucleotide 28 was used.

Lane 10: The reaction was performed in the same manner of lane 1 in which ~~without using~~ ologonucleotide 29 was used.

Lane 11: The reaction was performed in the same manner of lane 1 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 12: The reaction was performed in the same manner of lane 2 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 13: The reaction was performed in the same manner of lane 3 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 14: The reaction was performed in the same manner of lane 4 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 15: The reaction was performed in the same manner of lane 5 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 16: The reaction was performed in the same manner of lane 6 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 17: The reaction was performed in the same manner of lane 7 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 18: The reaction was performed in the same manner of lane 8 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 19: The reaction was performed in the same manner of lane 9 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Lane 20: The reaction was performed in the same manner of lane 10 in which ~~without treating~~ the reaction mixture was treated with heat at 80°C for 10 minutes.

Paragraphs beginning at Page 17, line 21 and ending at Page 18, line 34

~~Figure 12~~ Figures 12A-12C show the following: ~~Upper part~~ Figure 12A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide ~~consisting~~ containing sequence complementary to the DNA. "C" on the oligonucleotide indicates the relative position of mutation. ~~(A) in the middle part~~ Figure 12B is the photograph of detecting the signal of labeled oligonucleotide

that bound to the target DNA after triple strand DNA structure was electrophoresed. ~~(B) in the lower part~~ Figure 12C is the photograph of staining gel with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1: The reaction was performed in the same manner of lane 1 of ~~Figure 6~~ Figures 6B-6C of Example 6.

Lane 2: The reaction was performed in the same manner of lane 1 in which ~~without using~~ oligonucleotide 30 was used.

Lane 3: The reaction was performed in the same manner of lane 1 in which ~~without using~~ oligonucleotide 31 was used.

Lane 4: The reaction was performed in the same manner of lane 1 in which ~~without using~~ oligonucleotide 32 was used.

Lane 5: The reaction was performed in the same manner of lane 1 in which ~~without using~~ oligonucleotide 16 was used.

Lane 6: The reaction was performed in the same manner of lane 1 in which ~~without using~~ oligonucleotide 33 was used.

Lane 7: The reaction was performed in the same manner of lane 1 in which ~~without using~~ oligonucleotide 34 was used.

Lane 8: The reaction was performed in the same manner of lane 1 in which ~~without using~~ oligonucleotide 35 was used.

Lane 9: The reaction was performed in the same manner of lane 1 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 10: The reaction was performed in the same manner of lane 2 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 11: The reaction was performed in the same manner of lane 3 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 12: The reaction was performed in the same manner of lane 4 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 13: The reaction was performed in the same manner of lane 5 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 14: The reaction was performed in the same manner of lane 6 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 15: The reaction was performed in the same manner of lane 7 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Lane 16: The reaction was performed in the same manner of lane 8 in which ~~without treating~~ the reaction mixture was treated with heat at 70°C for 10 minutes.

Paragraphs beginning at Page 18, line 35 and ending at Page 19, line 32

~~Figure 13~~ Figures 13A-13B show the following: ~~Upper part~~ Figure 13A schematically shows the relation of the position between target DNA used for the experiment and the oligonucleotide ~~consisting~~ containing sequence complementary to the DNA. ~~(A) in the middle part~~ Figure 13B is the photograph of detecting the signal of labeled oligonucleotide that bound to the target DNA after triple strand DNA structure was

electrophoresed. ~~(B) in the lower part~~ Figure 13C is the photograph of staining gel with ethidium bromide after electrophoresis. Each lane is as follows:

Lane 1 and 2: The reaction was performed in the same manner of lane 1 of ~~Figure 1~~ Figures 1B-1C of Example 1.

Lane 3: The reaction was performed in the same manner of lane 1 in which ~~without using~~ oligonucleotide 36 that had insertion mutation was used.

Lane 4: The reaction was performed in the same manner of lane 1 in which ~~without using~~ oligonucleotide 16 that had mismatch mutation was used.

Lane 5: The reaction was performed in the same manner of lane 1 in which ~~without using~~ oligonucleotide 37 that had deletion mutation was used.

Lane 6: The reaction was performed in the same manner of lane 1 in which ~~without treating~~ the reaction mixture was treated with the heat at 70°C for 10 minutes.

Lane 7: The reaction was performed in the same manner of lane 2 in which ~~without treating~~ the reaction mixture was treated with the heat at 70°C for 10 minutes.

Lane 8: The reaction was performed in the same manner of lane 3 in which ~~without treating~~ the reaction mixture was treated with the heat at 70°C for 10 minutes.

Lane 9: The reaction was performed in the same manner of lane 4 in which ~~without treating~~ the reaction mixture was treated with the heat at 70°C for 10 minutes.

Lane 10: The reaction was performed in the same manner of lane 5 in which ~~without treating~~ the reaction mixture was treated with the heat at 70°C for 10 minutes.

Paragraph beginning at Page 20, line 7

In this invention, "polymorphism" means the individual difference in genome of identical species. The polymorphism consisting the difference of a nucleotide is preferred in this invention. Such "polymorphism" includes SNP (single nucleotide polymorphism) in which a nucleotide is inserted or deleted is also included.

Paragraph beginning at Page 26, line 11 and ending at Page 27, line 5

The present inventors revealed that there is the difference in stability to heat between the triple strand DNA in which the test polymorphic site in the target double strand DNA is complementary to the corresponding site in oligonucleotide probe and in which it is not complementary. When the test polymorphic site in the target double strand DNA is not complementary to the corresponding site in oligonucleotide probe, the oligonucleotide probe (described as "mismatch probe" hereafter) comprising the triple strand DNA can be released by the heat treatment under the suitable condition. The condition of temperature, in which oligonucleotide probe is not released from the triple strand DNA in which the test polymorphic site in the target double strand DNA is complementary to the corresponding site in oligonucleotide probe and is released from the triple strand DNA in which the site is not complementary to the corresponding site, varies depending on the length of target double strand DNA comprising the triple strand DNA, the length of the oligonucleotide probe, and their DNA nucleotide sequences, the extent of the complementation, and composition of reaction mixture (such as the concentration of

Tris). Optimal condition (such as composition of reaction mixture and temperature of heat treatment) can be suitably selected according to experiment and experience by one skilled in the art. Specifically, the condition indicated in Example 5 (~~Figure 6~~ Figures 6A-6C) can be used. Generally, when the concentration of Tris in the reaction mixture is decreased, the mismatch probe becomes easy to be released from the triple strand DNA. When the concentration of Tris is increased, the mismatch probe becomes hard to be released. Therefore, the concentration of Tris is preferred to be low when using long oligonucleotide probe while the concentration of Tris is preferred to be high when using short oligonucleotide probe.

Paragraph beginning at Page 28, line 34 and ending at Page 29, line 2

The above-mentioned kit of this invention can include nucleotide triphosphate, a buffer agent, and a reagent removing the homologous recombination protein. The ~~regent~~ reagent removing homologous recombination protein includes proteins such as ~~protein degradation enzymes~~ protein degradation enzymes and such.

Paragraph beginning at Page 29, line 19

~~Any patents, patent applications, and publications cited herein are incorporated by reference~~ Any patents, patent applications, and publications cited herein are incorporated by reference.

Paragraph beginning at Page 29, line 27 and ending at Page 30, line 13

The experiment was conducted to examine reaction components when triple strand DNA was formed. M13 mp18 RF DNA cut with restriction enzyme SnaB I to make it linear as target double strand DNA and 60 mer oligonucleotide 1 and 2 that have terminal sequence of the target DNA were prepared. pBR322 DNA cut with restriction enzyme Sca I to make it linear and 60 mer oligonucleotide 3 that has terminal sequence of the target DNA were prepared as target DNA. Oligonucleotide 1, 2, and 3 have the direction of sequence indicated as upper part of Figure ~~1~~ 1A. 5'-terminal of oligonucleotide 1 was labeled with ^{32}P using T4 polynucleotide kinase and [γ-^{32}P] ATP. Deproteinization was conducted by incubating 1 pmol labeled oligonucleotide 1, 3.0 μg RecA protein, 4.8 mM ATP-γS, and 200 ng target DNA with 20 mM magnesium acetate and 30 mM Tris acetate (pH 7.2) at 37°C for 30 minutes, adding 0.5% (W/Vol) SDS and 0.7 mg/ml proteinase K, and then incubating) at 37°C for 30 minutes. A half of the reaction mixture was electrophoresed with 1% agarose gel. The gel was stained with ethidium bromide, and the photograph of DNA was recorded. Gel was set on filter paper and was dried up in gel dryer. Autoradiogram of the gel was obtained, and signal from labeled oligonucleotide was recorded on X ray film. The result is shown in lane 1 of Figure ~~1 (A)~~ 1B. The nucleotide sequence of the oligonucleotides used were as follows:

Paragraph beginning at Page 30, line 20

Figure ~~1 (B)~~ 1C shows the result of recording the photograph of the DNA after staining the electrophoresed gel with ethidium bromide.

Paragraph beginning at Page 30, line 29 and ending at Page 31, line 2

We examined the orientation of oligonucleotide sequence necessary for the formation of triple strand DNA. M13 mp18 RF DNA cut with restriction enzyme SnaB I to make it linear as target DNA and 60 mer oligonucleotide 1, 2, 4, and 5 that have both terminal sequence of the target DNA were prepared. The oligonucleotide has the orientation of sequence indicated as upper part of Figure ~~2~~ 2A. The condition of reaction was same as Example 1. The result is shown in Figure ~~2 (A)~~ 2B. The nucleotide sequence of the oligonucleotides used were as follows:

Paragraph beginning at Page 31, line 7

Figure ~~2 (B)~~ 2C shows the result of recording the photograph of the DNA after staining the electrophoresed gel with ethidium bromide.

Paragraph beginning at Page 32, line 1

Figure ~~3 (B)~~ 3C shows the result of recording the photograph of the DNA after staining the electrophoresed gel with ethidium bromide.

Paragraph beginning at Page 32, line 11

The experiment to examine the length of oligonucleotide sequence necessary for the formation of the triple strand DNA was carried out. M13 mp18 RF DNA cut with restriction enzyme SnaB I to make it linear as a target DNA and 20-80 MER ~~80--20 mer~~ oligonucleotide that has terminal sequence of the target DNA were prepared. The condition of reaction was same as Example 1. The result is shown in Figure ~~4 (A)~~ 4B. The nucleotide sequence of the oligonucleotides used were as follows:

Paragraph beginning at Page 32, line 31

Figure ~~4 (B)~~ 4C shows the result of recording the photograph of the DNA after staining the electrophoresed gel with ethidium bromide.

Paragraph beginning at Page 33, line 6

We examined the thermostability of triple strand DNA. M13 mp18 RF DNA cut with restriction enzyme SnaB I to make it linear as a target DNA and 60mer oligonucleotide 1 that has terminal sequence of the target DNA were prepared. 5'-terminal of oligonucleotide 1 was labeled with ^{32}P. The reaction mixture contains 1 pmol labeled oligonucleotide 1,200 ng target DNA, 3.0 μg RecA protein, 4.8 mM ATP-γS, 30 mM Tris acetate (pH 7.2), and 20 mM magnesium. After the reaction mixture was incubated at 37°C for 30 minutes, 0.5 % (W/Vol) SDS and 0.7 mg/ml proteinase K was added to the mixture. Then, the mixture was incubated at 37°C for 30 minutes. After

phenol-chloroform extraction was performed once, unused oligonucleotide was removed by twice manipulation of S-400 spin column (Amersham Pharmacia Biotech). After the whole reaction mixture was treated with heat at 25°C for 10 minutes, half of that was electrophoresed with 1% agarose gel. After eletrophoresis, the gel was stained with ethidium bromide, and the photograph of DNA was recorded. Gel was set on filter paper and was dried up in gel dryer. Autoradiogram of the gel was obtained, and signal from labeled oligonucleotide was recorded on X ray film. The result is shown in lane 1 of Figure ~~6 (A)~~ 6(B).

Paragraph beginning at Page 33, line 27

Figure ~~6 (B)~~ 6C shows the result of recording the photograph of the DNA after staining the electrophoresed gel with ethidium bromide. Intensity of the signals from labeled oligonucleotide from lane 1 to lane 23 were measured with BAS2000 Image analyzer and the result is shown in Figure 7.

Paragraph beginning at Page 34, line 2

We examine the effect of the type of a nucleotide mutation in the oligonucleotide on the formation of triple strand DNA. The same reaction was conducted as lane 1 of Figure ~~6 (A)~~ 6B in Example 5 except using oligonucleotide 3 and PCR Product (a) as a target DNA. The result is shown in lane 1 of Figure ~~9 (A)~~ 9B. PCR reaction to prepare PCR Product (a) was conducted using 35-mer primer 1 that has the sequence same as the

terminal sequence produced by cutting pBR322 DNA with reaction enzyme Sca I and 35-mer primer 2 that has the sequence of another terminal of the DNA as primers and 1ng pBR322 DNA as template with 27 cycles of 98°C for 20 seconds and 68°C for 5 minutes following the general method. The part of the PCR product was electrophoresed with 1% agarose gel. The PCR Product (a) was extracted using QIAGEN Gel Extraction Kit and purified following the general method.

Paragraph beginning at Page 34, line 16

Figure ~~9 (B)~~ 9C shows the result of recording the photograph of the DNA after staining the electrophoresed gel with ethidium bromide. The nucleotide sequence of the oligonucleotides used were as follows:

Paragraph beginning at Page 35, line 10

The effect of the position of a nucleotide mutation in oligonucleotide to the target DNA on the sensitivity of detection of SNP was examined. M13 mp18 RF DNA cut with restriction enzyme SnaB I to make it linear as a target DNA and oligonucleotide that has terminal sequence of the target DNA and has a substituted nucleotide were prepared. Then, the effect of the position of a nucleotide mutation in oligonucleotide on the sensitivity of detection was examined. The result is shown in ~~Figure 10~~ Figures 10A-10C.

Paragraph beginning at Page 36, line 31

The result is shown in ~~Figure 12~~ Figures 12A-12C. It is revealed that the detection of SNP is possible regardless of the types and the position of the mutation.

Paragraph beginning at Page 37, line 1

M13 mp18 RF DNA cut with restriction enzyme SnaB I to make it linear as a target DNA and 60 mer oligonucleotide 1 that has terminal sequence of the target DNA were prepared. 5'-terminal of the oligonucleotide was labeled with ^{32}P. The reaction mixture contains 1 pmol labeled oligonucleotide 1,200 ng target DNA, 3.0 μg RecA protein, 4.8 mM ATP-γS, 30 mM Tris acetate (pH 7.2), and 20 mM magnesium acetate. After the reaction mixture was incubated at 37°C for 30 minutes, 0.5 % (W/Vol) SDS and 0.7 mg/ml proteinase K was added to the mixture. Then, the mixture was incubated at 37°C for 30 minutes. After phenol-chloroform extraction was performed once, unused oligonucleotide was removed by twice manipulation of S-400 spin column (Amersham Pharmacia Biotech). After the whole reaction mixture was treated with heat at 25°C for 10 minutes, half of that was electrophoresed with 1% agarose gel. After electrophoresis, the gel was stained with ethidium bromide, and the photograph of DNA was recorded. Gel was set on filter paper and was dried up in gel dryer. Autoradiogram of the gel was obtained to detect signal which was recorded on X ray film. The result is shown in lane 1 of Figure ~~13 (A)~~ 13B.